AD-A165 448 EFFECTS OF DREDGED MATERIALS ON ZOOPLANKTON(U) OLD DDOMINION UNIV NORFOLK VA APPLIED MARINE RESEARCH LAB R W ALDEN ET AL. JUN 84 DACW65-81-C-0051 1/1

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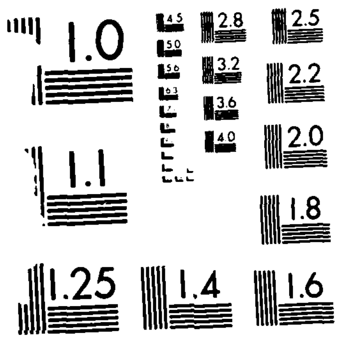

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APPLIED MARINE RESEARCH LABORATORY
OLD DOMINION UNIVERSITY
NORFOLK, VIRGINIA

EFFECTS OF DREDGED MATERIALS ON ZOOPLANKTON

By

Ray W. Alden, Principal Investigator

and

Renee S. Crouch

Prepared for
Department of the Army
Norfolk District, Corps of Engineers
Norfolk, Virginia 23510

Under
Research Contract DACW65-81-C-0051
Work Order No. 16

June 1984

US Army Corps
Of Engineers
Norfolk District

Report B- 15

Unclassified
SECURITY CLASSIFICATION OF THIS PAGE

AD-A165 448

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release, distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S) B-15
6a. NAME OF PERFORMING ORGANIZATION Old Dominion University, Applied Marine Research Lab. 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION U.S. Army Corps of Engineers, Norfolk District
6c. ADDRESS (City, State, and ZIP Code) Norfolk, Virginia 23508	7b. ADDRESS (City, State, and ZIP Code) Norfolk, Virginia 23510-1096
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Corps of Engineers, Norfolk District 8b. OFFICE SYMBOL (If applicable) NAOPL; NAOEN	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DACW65-81-C-0051
8c. ADDRESS (City, State, and ZIP Code) Norfolk, Virginia 23510-1096	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Effects of Dredged Materials on Zooplankton

12. PERSONAL AUTHOR(S)
Alden, R.W. III, and R. S. Crouch

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM ______ TO ______	1984, June	20

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	zooplankton, dredged material effect, copepod, biossay, acute toxicity, suspended particulates, open ocean disposal, Southern Branch Elizabeth River, sediment quality, Acartia tonsa

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Elutriate bioassays with Acartia tonsa suggest that sediments from the Southern Branch lower reaches could significantly impact the zooplankton community. The intermittent nature of disposal operations and the small size of the disposal site compared to the size of regional larval transport routes and spawning grounds evokes little concern for open ocean disposal of any but the most contaminated sediments.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Craig L. Seltzer	22b. TELEPHONE (Include Area Code) (804) 441-3767/827-3767 22c. OFFICE SYMBOL NAOPL-R

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

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OLD DOMINION UNIVERSITY
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Work Order No. 16

Submitted by the
Old Dominion University Research Foundation
P.O. Box 6369
Norfolk, Virginia 23508

June 1984

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EFFECTS OF DREDGED MATERIALS ON ZOOPLANKTON

By

Ray W. Alden[1] and Renee S. Crouch[2]

ABSTRACT

A study was conducted to determine the potential impact of open ocean disposal of sediments dredged from a highly industrialized seaport. Sediments from three potential dredge sites along the Southern Branch of the Elizabeth River, Hampton Roads, Virginia were evaluated for acute toxicity to the calanoid copepod, Acartia tonsa Dana. The suspended particulate fractions of dredged materials were tested in a series of 96-hour, static bioassays. Significant mortalities were observed for all three sites with the sediments from mortality. Analysis of results indicates that mortality was correlated with the total volatile residue portion of the suspended particulate load and to site-specific factors. Potential effects on the copepod community were evaluated in reference to dispersal of suspended particulates in the open ocean.

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INTRODUCTION

Hampton Roads and the surrounding estuarine systems of Tidewater, Virginia, provide the setting for one of the most heavily industrialized coastal areas on the eastern seaboard. It is also the site of the largest military port in the world. The necessity of construction and maintenance of channels in the harbor raises the question of where the spoils of dredging operations can be disposed with minimal ecological impact. A great deal of research has been directed toward evaluating the potential impacts and viability of open ocean disposal (Pequegnat et al., 1978; Alden & Young, 1982). The deep ocean already assimilates the millions of tons of river borne sediments brought to it each year, and its capacity for handling additional sediment is apparently very great. In developing guidelines for selection of suitable disposal areas, an assessment of potential biological effects is necessary.

Previous dredged material studies have produced varied results depending upon the sensitivity of the test organism studied and the methods of analysis used. Early studies by Hoss et al. (1974) with larval fish and DeCoursey and Vernberg (1975) using three larval zooplankters showed significant lethal effects to be associated with extracts of sediments from Charleston Harbor, S.C. However, more recent studies done by several researchers on a variety of seaport systems have indicated that the effects of dredged sediments are generally minimal (Lee et al., 1975; Peddicord et al., 1975; Lee and Jones, 1977; Hirsch et al., 1978; and Alden and Young, 1982). New elutriation procedures which recommend the use of compressed air to mix sediments and water may contribute to lower toxicity. Lee et al. (1975) and Hirsch et al. (1978) reported that toxins commonly observed in

sediments appear to exhibit low bioavailability under the oxidizing conditions typically found at open ocean disposal sites. In addition, the degree of toxicity of dredged sediments varies with the sensitivity of the test organism. Results of Lee *et al*. (1975), Peddicord *et al*. (1975), the Tereco Corp. (1978), and McFarland and Peddicord (1980) showed soft, muddy bottom organisms to be quite tolerant of dredged materials; open water fish, fouling organisms, and sandy bottom epifauna to be intermediate in tolerance; and larval zooplankton to be the least tolerant.

Zooplankton are highly sensitive to changes in water quality and have been recommended for the evaluation of the effects of suspended particulates which are introduced into the water column during periods of disposal of dredged material. The zooplankton community is a major link in most estuarine food webs, playing the vital role of primary consumers in the energy transfer to higher trophic levels. Copepods are generally regarded as representing the dominant zooplankton group in the marine and estuarine environs (Conover, 1956).

The present study involves an assessment of the potential biological effects of open ocean disposal of sediments from dredging sites along the Southern Branch of the Elizabeth River. Three composite sites were evaluated through suspended particulate phase bioassays using the calanoid copepod, *Acartia tonsa* Dana, as the test organism.

MATERIALS AND METHODS

Study Area

The Port of Hampton Roads, Virginia, is located at the mouth of the Chesapeake Bay (Figure 1). The Elizabeth River is the principal deepwater channel in this seaport surrounded by the major metropolitan area that

includes the cities of Norfolk, Portsmouth and Chesapeake. The Elizabeth River is composed of three branches, the Western, Southern and Eastern. In the vicinity of the Western and main branch confluence, is located a primary sewage treatment plant, a chemical company, two seafood packing plants and a major coal loading facility. The most highly industrialized areas of the River are found in the inner harbor just south of the confluence. In this area are the Norfolk Naval Shipyard, steel works, metal plating industries, two creosote plants, four oil depots, as well as other industries. The Southern Branch is the least developed with a surrounding area that includes a power generating plant and a few fertilizer and grain plants, most of which have been abandoned.

Nine stations along the Southern Branch of the Elizabeth River were selected to assess the potential lethal effects of open ocean disposal of dredged materials. Composite samples were made by combining sediments from three sets of stations separated by 1 mile intervals to form sites 1, 2, and 3, respectively. Composite site 1 represents the area of the confluence of the Western Branch with the main branch of the Elizabeth River. Site 2 incorporated the inner harbor, and site 3 represented the area with the least development control or reference sediments in the toxicity tests. Sediments were collected from the non-industrialized Rudee Inlet, Virginia Beach.

Toxicity Testing Methods

Methods recommended by the U. S. Environmental Protection Agency and the U. S. Army Corps of Engineers (EPA/COE, 1978) were followed during collection of sediments, preparation of dredged materials, suspended particulate bioassays, and cleaning of glassware.

Sediments were collected at each of the sites along the Elizabeth River using a stainless steel bucket grab. Sediments from Rudee Inlet were

collected manually at low tide from three subtidal areas. All samples were stored at 4°C for periods of approximately two weeks until the bioassays were performed.

Collection, sorting and handling of test organisms were done according to the procedures in Standard Methods (APHA, 1976). Collections were made between mid April and early June, 1983, at Long Creek Bridge, east of Lynnhaven Inlet in Virginia Beach (Figure 1). During the collection period water temperatures ranged from 13.7 to 22.5°C, salinities fluctuated between 16.4 and 20.1 $^{o}/oo$ and the average dissolved oxygen level was 8.50 mg/l.

The copepods were acclimated gradually to the experimental conditions of 20°C and 20 $^{o}/oo$ salinity in a controlled environmental chamber over a 24 hour period. With the exception of the initial temperature in April, changes in temperature and salinity did not exceed 3°C and 3 $^{o}/oo$. Estuarine water was gradually replaced with artificial seawater and holding containers were aerated.

Adult copepodi, free from obvious parasites, deformities, or injuries, were sorted under dissecting microscope utilizing a wide bore pipette. All experiments were begun immediately after the sorting and counting of copepods was completed. The elapsed time from collection to start of the bioassays was approximately 24 to 36 hours.

In preparation for the tests, 300-ml glass Erlenmeyer flasks were analytically cleaned for use as test vessels. Oxygen concentrations in the test flasks were maintained at saturation levels throughout the experiments by aeration supplied through an oil-free air compressor, valves and Pasteur pipets.

To prepare the suspended solid elutriate, equal volumes of sediments from each of three stations per site were mixed manually to ensure homogen-

ous composite samples. Elutriation of the dredge spoils was begun by vigorously agitating a 1:4 (v/v) mixture of composite sediments in artificial seawater in an acid washed polyethylene barrel with a compressed air "wand" for 30 minutes. The resulting suspension was allowed to settle for one hour and the supernatant or "elutriate" was siphoned into an analytically cleaned 20 liter plastic bucket. The suspended solid phase bioassays were begun immediately.

Elutriate was added to each of the flasks in volumes sufficient to give a total volume of 200 ml and the following concentrations of elutriate in the artificial seawater: 100%, 50%, 25%, 10%, and 0% (control). Replicates of each experimental concentration were set up with ten copepodi per test flask. The large amount of particulates in the test flasks containing the elutriate prevented direct observation of the copepods to determine accurate numbers of live organisms. The procedure for vital staining of live copepods was modified from the work of Dressel et al. (1975). To facilitate counting the live copepods, a serial sacrifice series was set up. At each time interval, three replicates of each concentration were stained for 30 minutes with a one percent neutral red solution and were preserved in ten percent buffered formalin. Neutral red was taken up only by the tissues of live copepods. Samples were stored at 4°C until counting was done. The stained and preserved samples were acidified immediately before counting with a 1:1 (v/v) solution of 5N acetic acid and 5N sodium acetate and those copepodi which were alive when the neutral red was added turned a bright magenta while those which were dead remained a light pink or white. Counts using the staining and serial sacrifice were made at 2, 4, 8, 12, 24, 48, 72, and 96 hours after the experiments were begun.

Total suspended matter (total nonfiltrable residue) and total volatile

and fixed residues were determined for all concentrations of suspended solid elutriate from the four sediment test sites.

Statistical Analysis

The traditional method used to analyze mortality observed during a bioassay involves the calculation of LC50 values. Recent studies (Alden and Young, 1982) favored the use of the William's Test (1971, 1972) for dredged material toxicity tests. Furthermore, data from the present study did not meet the requirements for determination of LC50's. Mortalities at 96 hours from sites 1, 2, and 3 were greater than 50% at all but control concentrations, while mortalities in site 4 tests did not exceed 50% in the 100% elutriates. The data from the suspended solid bioassays were statistically evaluated for each counting period at each site. The experimental and control survivorship data were tested for homogeneity of variances by Cochran's C and Bartlett-Box F tests. All data were found to be homogeneous and normally distributed and met the assumptions for parametric statistical analysis. A one-way Analysis of Variance (ANOVA) was done to determine whether there were significant differences ($\alpha = 0.05$) in the numbers alive between the control and various elutriate concentrations at each time period for each site. Additional ANOVA's were performed to determine if there were significant differences in the numbers alive comparing the different experimental sites at each concentration for each time interval. A William's test was performed following each of the ANOVA's to determine which concentrations produced mortalities which were significantly higher than the control levels.

A multiple regression computer program was used to examine the relationships between the number of live copepods at the 96-hour time interval and total suspended matter (TS), total volatile residue (TVR) (both loga-

rithmically transformed), the responses unique to each of the sites (dummy variables) and all appropriate interactions between independent variables. Inspection of a scatter plot of the residuals confirmed a linear regression relationship and homogeneity of variance. Significant regression coefficients for the dummy variables would indicate that factors unique to each site explain effects on survival (see Kim and Kohout, 1975, for a discussion of dummy regression variables).

RESULTS

Data from the ANOVAs testing lethal effects in the toxicity tests are presented graphically by site (Figure 2). Percent mortality values are plotted (mean values with standard error bars) against the hour of experiment for each elutriate concentration. Mortality levels which were found by the ANOVA and William's test to be significantly higher than control values are indicated by closed circles, while control values are represented by open circles.

It is apparent that the elutriate from each site produced significant lethal effects by 96 hours. The suspended solid tests of the reference site (site 4) resulted in lower mortalities, only 50% in the full strength (100%) elutriate. Moreover, the site 4 mortality levels were not significant until the 96-hour time interval (Figure 2d). All other elutriates produced 100% mortality in the 100% concentration by the end of the experiments. Mortalities from the site 2 tests were significantly elevated early in the experiments (at 2 hours or 4 hours depending on concentration) and were much greater in magnitude than those observed from the suspended solid phases of sites 1, 3, or 4 (Figure 2b). Elutriates from sites 1 and 3 produced

mortality-time curves which were somewhat comparable in magnitude and in the time (48 hours) at which mortalities first became significantly different from controls (Figures 2a,c).

Multiple regression analysis was used to determine which independent variables explained a significant percentage of the variance in the data and whether interactions between these variables existed (Table 1). Variables were added to the analysis in a stepwise manner and in order of decreasing significance. The logarithm of the total volatile residue (TVR) explained 71% of the variance in numbers alive (Table 1, $R^2 = 0.71$). The dummy variable for site 4 explained an additional 18% of the variance due to responses unique to the reference site in addition to the TVR. Finally, a small but significant percentage of the variance was explained by the interaction of the log TVR with the particular responses at site 2. The total R^2 value of 0.90 indicated that the three variables mentioned explained 90% of the variance in numbers of copepods surviving after 96 hours. The regression relationship was highly significant at the $\alpha = 0.001$ level of probability. Table I also presents regression equations which model survivorship for each of the sites. Site 4 elutriates produced less mortalities than the other tests, across the TVR range. On the other hand, the site 2 tests produced lethal effects which were progressively more toxic at higher TVR levels. In fact, the slope of the relationship is somewhat conservative due to the fact that mortalities reach the 100% level at TVR levels below the maximum values.

DISCUSSION

Relationship to Previous Dredged Material Bioassay Studies

The total contaminant content of the sediment is not a reliable basis

for developing dredged material disposal criteria. No correlations have been found between the content of contaminants in dredged materials and their release and toxicity at the disposal site (Lee and Jones, 1977; and Hirsch et al., 1978). Therefore, site-specific bioassays are needed to assess the magnitude of lethal effects that might be expected for test organisms exposed to various sediment fractions.

Suspended solid elutriates from all three sites in the Southern Branch of the Elizabeth River were found to be highly toxic to *Acartia tonsa.* Average mortalities at the 96-hour interval for populations exposed to sediments from sites 1, 2, and 3 were 95, 100, and 100%, respectively. Exposure to sediments from the reference site resulted in a moderate 96 hour average mortality value of 50%. Mortalities from Elizabeth River sediments were significantly higher than reference sediment mortalities at all sites. Sediments producing the greatest effects were collected from site 2 as expected from the heavily industrialized region of the River. Thus the degree of nearby industrial development seems to be qualitatively related to mortalities observed in the bioasssays. This observation was also made by Alden and Young (1982) in their evaluation of sediment from the Port of Hampton Roads employing bioassays of the grass shrimp *Palaemonetes pugio*.

Several mortality patterns are noted in the bioassay results. The length of time to which test organisms were exposed to potential toxins in elutriate concentrations were not significantly different from controls during the first 24 to 48 hours of exposure. During the last 24 hours of the experiments, however, there was less difference between mortalities in 100 and 10% elutriates than between 10% elutriates and controls. This suggests that prolonged exposure to even low amounts of the suspended solid

phase may be as important as the concentrations to which they are initially exposed. The concentration of elutriate did have some effects on overall mortalities. There are significant differences in lethality between concentrations, although the effects are not linear; i.e., the 10% elutriate results in more than one-tenth of the mortality noted in the 100% elutriate.

Fine particulates have a greater surface area allowing for a greater number of sites for adsorption of contaminants. The smaller sized particles also remain in suspension longer than the larger ones. Organic material associated with natural sediments may also absorb or complex contaminants, as will iron and manganese oxides and hydroxides that exist as discrete particles or fine coatings (McFarland and Peddicord, 1980). Contaminants are also dissolved in the interstitial water (Brannon et al., 1976). All of the above factors complicate the assessment of the toxicity of the suspended particulates and associated pollutants. The same study area was assessed by Alden and Young (1982) who demonstrated that lethal effects were associated primarily with the fine suspended solid materials. Analysis of total suspended matter in the present study also singles out suspended particulates in particular the volatile or organic component of these as the factor explaining the majority of the variance in Acartia tonsa survival during bioassays of sediments from the four test sites. Shuba et al. (1978) also assessed the lethality of several test sediments on A. tonsa. Their results were variable, with mortalities ranging from little to highly significant. Acartia were shown to be sensitive to heavy metals associated with sediments.

Mechanical or abrasive action of suspended particles may also have contributed to the death of copepods in the present study. There was a significant lethal effect by the reference sediment compared with controls.

The small size of the test organisms and their relative sensitivity in comparison with larger, more hardy test organisms, such as grass shrimp, increases their potential susceptibility. Therefore, the detrimental characteristics of the suspended particulates which show up in the analysis as site-specific factors may be the result of the interaction between the toxic action of associated chemical contaminants, especially at heavily industrialized sites, and the physical stress of the high suspended solid load.

Assessment of Water-Column Impacts

Due to the dynamic nature of the marine environment, materials dumped will be dispersed, mixed, and diluted. Therefore, the initial mixing expected at the disposal site must be evaluated to relate the suspended solid bioassays to the natural environment. Initial mixing is defined in the Implementation Manual (EPA/COE, 1978) as the dispersion of the suspended particulate phase that occurs within four hours after disposal. A concentration of 1% of the elutriate concentration producing significant toxic effects may not be exceeded beyond the boundaries of the disposal site within the four hour initial mixing period nor at any point including the dump site after the initial mixing. Initial mixing calculations were done using the hypothetical release zone method suggested in the Manual. This method uses information on the characteristics of the disposal vessel and on the depth of the site to calculate the volume of the mixing zone and the volume of material to be dumped. The example presented in the Implementation Manual assumed a 90% silt/clay value and the sediments in the present study also appeared to have a high silt/clay content. The model is fairly conservative, as it does not take into account dispersal by currents and waves so the actual concentrations would likely be less. According to the calculations used in the model, the concentration at the disposal site after

initial mixing of four hours was only 0.06% of the original concentration (Alden and Young, 1982). Since the predicted concentration of dredge spoils determined in the model is close to but less than 1% of the toxic concentration at four hours for site 2, it does not violate the guidelines established in the Implementation Manual and would be acceptable for open ocean disposal. It should be noted, however, that the two hour concentration from sediments at site 2 was greater than 1% of the toxic concentration, although this is considered acceptable within the four hour time period following the dump.

The close proximity of the dilution curve to the maximum allowable toxic concentration at the four hour interval raises the question of the acceptability of sediments from site 2. If a 6% concentration of the original elutriate had produced significant toxicity at the four hour count, then the dredged materials would no longer be acceptable for open ocean disposal according to the established guidelines. Since a 6% or lower elutriate concentration was not tested for lethality, the question of acceptability of sediments from site 2 is purely speculative. However, the significant mortalities in the 10% elutriate at four hours suggest that sediments from the most heavily industrialized area could significantly impact the zooplankton community.

The magnitude of the impact of dredged material disposal must not only be assessed in reference to dilution effects but must include characteristics of the zooplankton community itself. Even with the "worst case" sediments, such as from site 2 of the present study, would a 30% mortality of zooplankton in the vicinity of the disposal have long-term significant effects? Zooplankton are renowned for their high turnover rates; the average adult life span of *Acartia tonsa* at 20°C is 30 days with individuals

reaching maturity within 16 to 17 days (APHA, 1976). In addition, if disposal operations are intermittent, then minimal effects would be expected. Thus, complete loss of a plankton community in the small mixing zone area would be likely to produce only a temporary effect, as populations are rapidly replaced.

A variety of sublethal effects have been observed when zooplankton are subjected to suspended particulates. DeCoursey and Vernberg (1975) reported depressed respiration rates and depressed swimming activity in larval zooplankton. Sherk _et al_. (1976) noted that _A. tonsa_ exhibited biologically significant reductions in ingestion at all concentrations of suspended particle types tested. The effects increased with increasing concentration. Furthermore, Paffenhofer (1972) reported serious problems of nutrition, growth, survival and reproduction when _Calanus helgolandicus_ was exposed to suspensions of "red mud" (mostly iron oxide). Copepods were weakened and not able to make their characteristic sudden escape movements because of decreased ingestion of phytoplankton cells when red mud was present. They ingested large quantities of particles but did not obtain sufficient nutrients to develop as well as controls (a decreased growth rate was observed). In addition, ovary development in females was much decreased because of insufficient energy reserves. Thus, prolonged exposure to sublethal levels of suspended particulates may have adverse effects on nutrition, growth and reproduction of zooplankton resulting in the "ecological death" of the community. A significant reduction in the zooplankton standing stock could be detrimental to larval and juvenile stages of important benthic and nektonic species (Sherk _et al_., 1976 and Pequegnat _et al_., 1978). In addition, extreme sensitivity of juvenile stages to suspended solids has been demonstrated.

Presumably, potential lethal and sublethal impacts could be minimized with intermittent disposal operations, especially if the disposal site were small compared with the size of regional larval transport routes and spawning grounds. These considerations are particularly relevant for the Norfolk Disposal Site since the disposal operations are expected to occur only four times per day and test dumps have indicated that the area of impact would be less than 300 m from the point of disposal (Darby *et al*., 1981). However, considering the potential long-term lethal and sublethal effects of contaminated dredged materials on zooplankton in general and meroplankton in particular, it may be environmentally sound to avoid any ocean disposal of materials from the area shown to be most toxic to *Acartia tonsa* (i.e. from the region surrounding site 2 on the Southern Branch of the Elizabeth River).

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Table I. Multiple regression table for *Acartia tonsa* survival.

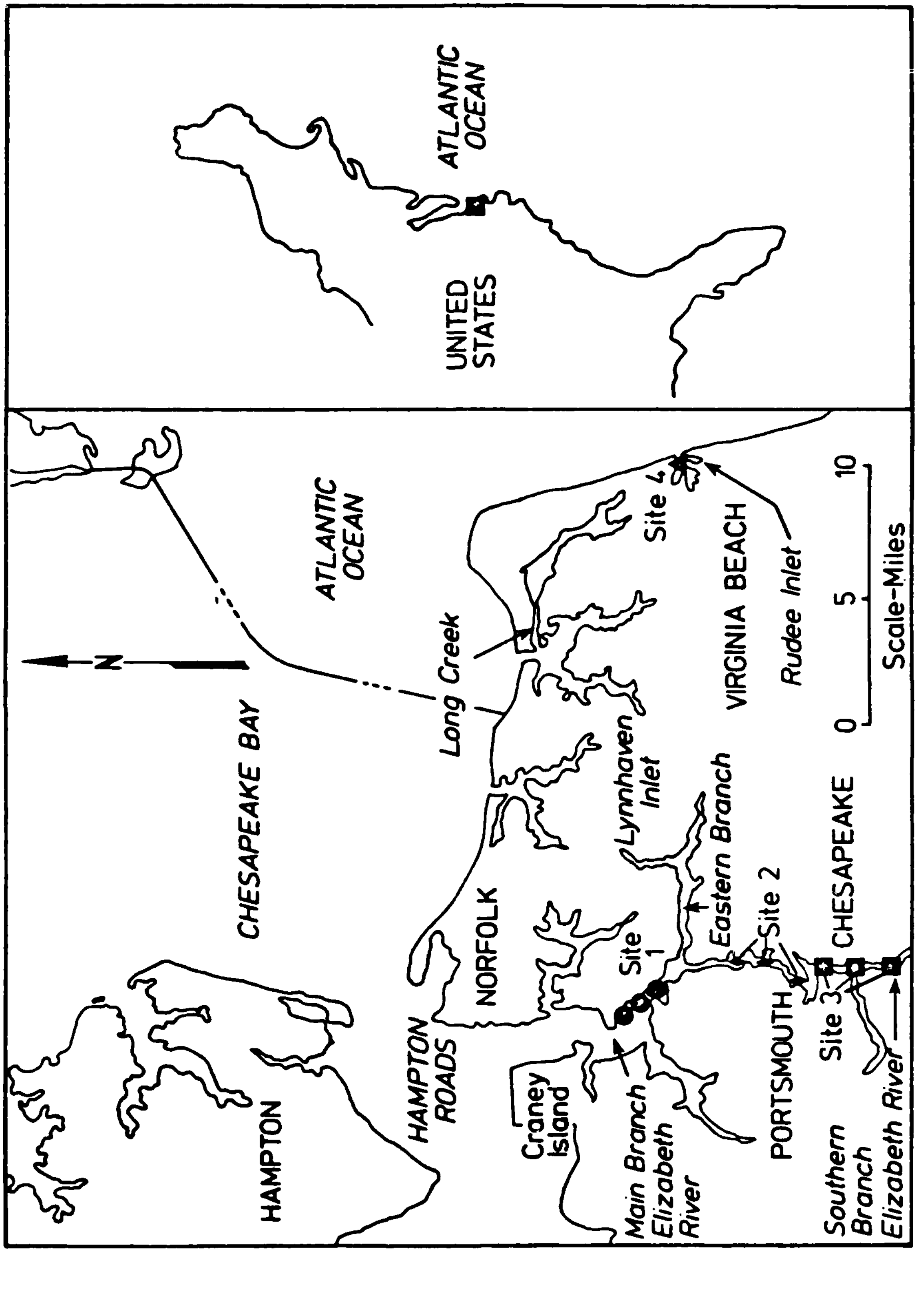
Source of Variation	R^2	Coefficient	F	d.f.	F Probability
Log Total Volatile Residue (TVR)	0.71	- 4.22	300.17	1,56	0.001
Reference Site (site 4)	0.18	2.73	78.04	1,56	0.001
Interaction term TVR and site 2	0.01	- 0.43	6.53	1,56	0.001
Constant	--	10.22	--	--	--
Total	0.90	--	168.01	3,56	0.001

Regression Models for Survival.

Category	Equation	Predicted # Alive at Mean TVR*
Reference (Site 4)	# Alive=10.22-(4.22 x log TVR)+ 2.73	4.72
Interaction TVR-Site 2	# Alive=10.22-(4.22 x log TVR)-0.43 x log TVR)	1.15
Sites 1,3	# Alive=10.22-(4.22 x log TVR)	1.99

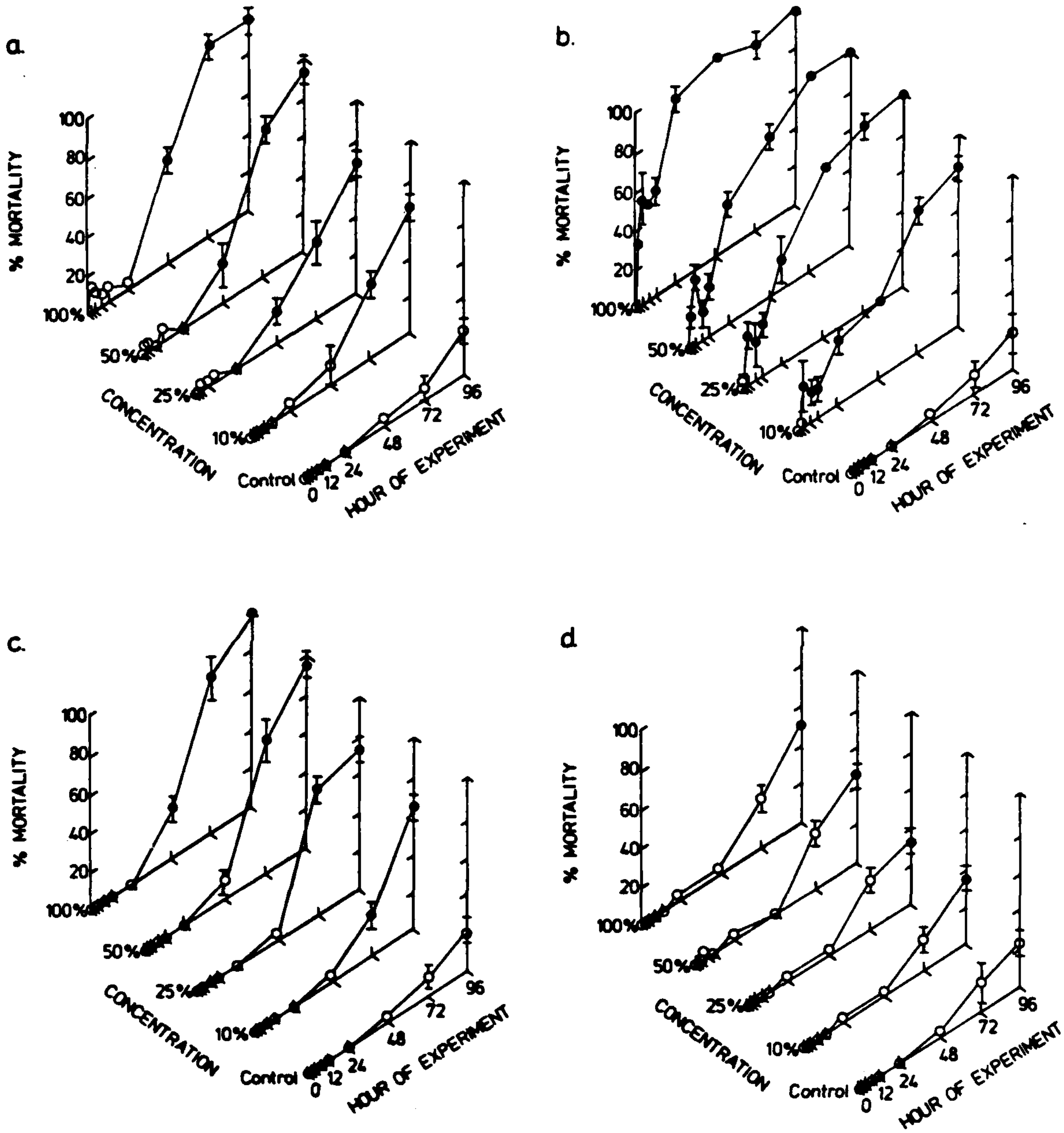
*Mean TVR = 89.317 mg/1

Figure 1. Study area. Collection sites 1-3 are in the Elizabeth River, while site 4 (reference) is in Rudee Inlet. The test organisms were collected on Long Creek of the Lynnhaven estuarine system.



HAMPTON
CHESAPEAKE BAY
ATLANTIC OCEAN
N
HAMPTON ROADS
NORFOLK
Long Creek
Lynnhaven Inlet
VIRGINIA BEACH
Rudee Inlet
Site 4
Site 1
Site 2
Eastern Branch
Craney Island
Main Branch Elizabeth River
PORTSMOUTH
Site 3
CHESAPEAKE
Southern Branch Elizabeth River
0
5
10
Scale-Miles
UNITED STATES
ATLANTIC OCEAN

Figure 2. Relationships between concentration, hour of experiment, and percent mortality of *Acartia tonsa* exposed to suspended solid elutriates of sediments collected from: a) site 1; b) site 2; c) site 3; and d) site 4. Solid circles represent mean mortalities shown to be significantly different from controls, while open circles represent values not shown to be significantly different from controls. Vertical lines are two standard errors of the means.


a.
b.
c.
d.
% MORTALITY
100
80
60
40
20
100%
50%
25%
10%
Control
CONCENTRATION
0
12
24
48
72
96
HOUR OF EXPERIMENT

DTIC
FILMED
4-86
END